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International Journal of Obesity (2001) 25, 33–38
 0307–0565/01 $15.00
www.nature.com/ijo

PAPER

The effect of an increased free fatty acid concentration on thermogenesis and substrate oxidation in obese and lean men

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OBJECTIVE: To examine whether a certain increase in plasma free fatty acid (FFA) concentration leads to similar increases in lipid oxidation and energy expenditure in obese and lean men.
DESIGN: The study protocol consisted of a 30 min baseline period after which subjects received an i.v. bolus of 1000 IE heparin. Then consecutive infusions of 4.9, 9.8 and 19.6 μl/kg fat-free mass (FFM) · min of a lipid heparin mixture were started, each infusion for 30 min.
SUBJECTS: Eleven obese and 13 lean men with a mean body mass index (BMI) of 34.2 ± 1.0 (± s.e.m.) and 23.9 ± 0.5 kg/m^2 and age 46.0 ± 1.0 and 42.6 ± 1.5 y, respectively.
MEASUREMENTS: Energy expenditure, respiratory exchange ratio (RER) and carbohydrate and lipid oxidation were continuously measured by indirect calorimetry. At the end of each infusion period, a blood sample was taken for FFA, glycerol, insulin, β-hydroxybutyrate, noradrenaline and adrenaline determination.
RESULTS: At baseline, plasma FFA levels were comparable in both groups. Lipid heparin infusion increased plasma FFA concentration by 301 ± 47 μmol/l and 332 ± 27 μmol/l in obese and lean men. Energy expenditure increased similarly in obese and lean men (0.34 ± 0.08 *vs* 0.40 ± 0.08 kJ/min, NS) during lipid heparin infusion, whereas RER decreased similarly in both groups. Lipid oxidation rates were comparable at baseline and increased similarly in obese and lean men (19 ± 5 *vs* 13 ± 4 mg/min, NS). Baseline plasma insulin levels were higher in the obese, but did not change during lipid heparin infusion. Plasma β-hydroxybutyrate concentrations were similar at baseline, but increased significantly less in the obese during lipid heparin infusion. Baseline noradrenaline and adrenaline concentrations did not differ significantly between groups. During lipid heparin infusion, plasma noradrenaline levels decreased significantly, but plasma adrenaline levels remained unchanged in both groups.
CONCLUSION: A certain increase in plasma FFA concentration leads to similar increases in lipid oxidation and energy expenditure in obese and lean men. The accumulation of fat in obese subjects may therefore be more likely to be due to a defect in adipose tissue lipolysis than a defect in lipid oxidation.
International Journal of Obesity (2001) **25**, 33–38

Keywords: energy expenditure; lipid oxidation; obesity

Introduction

Energy expenditure increases after the ingestion or infusion of nutrients. Activation of the sympathetic nervous system (SNS) may, at least partly, contribute to this increase in thermogenesis,[1–3] although this finding is not consistent.[4] Previous studies have shown that mainly β_1- and β_2-adrenoceptors are involved in sympathetically mediated thermogenesis.[5,6] During selective β_1-adrenergic stimulation, lipolysis, lipid oxidation and energy expenditure increase.[7,8] Free fatty acids (FFA), needed for lipid oxidation, are released from the adipose tissue by stimulating its β_1-adrenoceptors. The increase in lipid oxidation and thermogenesis is assumed to be localized predominantly in skeletal muscle,[9,10] but this tissue contains mainly β_2-adrenoceptors

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Received 8 October 1999; revised 24 July 2000; accepted 20 September 2000

and presumably no β_1-adrenoceptors.[11] β_1-Adrenergic stimulation is therefore not likely to increase lipid oxidation by direct stimulation of skeletal muscle. Another possibility is that the availability of FFA in the blood may induce the increase in lipid oxidation and energy expenditure.

Furthermore, it is suggested that the thermogenic effect of food is reduced in obese subjects, although the results are inconsistent (for review see De Jonge *et al*[12]). A reduction in diet-induced thermogenesis in the obese might be explained by their reduced thermogenic response during SNS stimulation.[13,14] Blaak *et al*[10] showed that obese men have an impaired response of lipolysis and lipid oxidation during isoprenaline (non-selective β-agonist) infusion. If the availability of FFA in blood is a limiting factor for thermogenesis, the reduced lipolytic response in obese subjects could explain their reduced increases in lipid oxidation and thermogenesis.

On the other hand, thermogenesis and lipid oxidation could be impaired due to a defect in skeletal muscle metabolism. Colberg *et al*,[15] showed that women with visceral obesity have reduced FFA utilization in muscle in the post-absorptive state. Furthermore, Colberg *et al*[15] and Simoneau *et al*[16] found that obese women have a decreased oxidative capacity and increased glycolytic and anaerobic capacities of skeletal muscle. This may suggest that not FFA availability in blood, but rather the oxidative enzymes in skeletal muscle may be the limiting factor for the increase in lipid oxidation and thermogenesis.

The aim of the present study was to examine whether a certain increase in plasma FFA concentration leads to similar increases in lipid oxidation and energy expenditure in obese and lean men.

Material and methods

Subjects

Eleven obese and 13 lean male volunteers participated in this study. The physical characteristics of the subjects are summarized in Table 1. All subjects were in good health as assessed by medical history and physical examination. Furthermore, both lean and obese subjects spent no more than 2 h a week in organized sports activities. The study protocol was reviewed and approved by the Ethics Committee of Maastricht University and all subjects gave informed consent before participating in the study.

Table 1 Subject characteristics

Parameter	*Obese men*	*Lean men*
Body weight (kg)	106.9±3.0*	75.2±2.9
Height (m)	1.77±0.02	1.77±0.02
BMI (kg/m^2)	34.2±1.0*	23.9±0.5
Body fat (%)	32.4±1.5*	19.1±1.6
Fat-free mass (kg)	72.1±2.0*	60.7±2.1
Age (y)	46.0±1.0	42.6±1.5

Results are mean±s.e.m. for 11 obese and 13 lean men. Unpaired *t*-test: *$P < 0.001$.

Experimental design

Subjects arrived at the laboratory at 8:00 am after an overnight fast. They came by car or by bus to minimize the amount of physical activity before the test. On arrival, one canula was inserted into a forearm vein to infuse a lipid heparin mixture and to sample venous blood. A second canula was inserted into a dorsal hand vein of the contralateral arm. This hand was kept in a hotbox with a temperature of 60°C for the sampling of arterialized blood. Ventilated hood measurements were started with the subject in supine position and room temperature was kept between 21 and 23°C. After a 30 min baseline measurement, a bolus of 1000 IE heparin (Leo Pharmaceutical Products, Weesp, The Netherlands) was given, after which a continuous infusion of increasing doses of 4.9,9.8 and 19.6 µl/kg FFM · min of a lipid heparin mixture (Intralipid® 20%, Pharmacia, Woerden, The Netherlands) (1000 IE heparin per 100 ml Intralipid) was started, each dose given for 30 min. At the end of each 30 min period, a blood sample was taken. In a pilot study, blood samples were taken after 20, 25 and 30 min in each infusion period. The coefficient of variation (CV) for plasma FFA concentration within each set of three samples was 5%. Therefore, it was assumed that steady state was achieved in plasma FFA concentrations at the end of each infusion period.

Clinical methods

Body density was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000, Mijnhardt, Bunnik, The Netherlands). Body composition was calculated according to the equation of Siri.[17] Whole body energy expenditure and respiratory exchange ratio (RER) were measured by an open-circuit ventilated hood system (Oxycon beta, Mijnhardt, Bunnik, The Netherlands). O_2 consumption (CV:3.0%) and CO_2 production (CV:1.7%) values were averaged over the last 10 min of each 30 min period. Energy expenditure was calculated according to the formula proposed by Weir.[18] Carbohydrate and lipid oxidation rates were calculated as described by Ferrannini,[19] assuming that protein oxidation accounted for 15% of total baseline energy expenditure and remained constant during the remainder of the test.

Analytical methods

Arterialized blood samples for the determination of FFA, glycerol, β-hydroxybutyrate and insulin were preserved in sodium-EDTA. Venous samples for the determination of noradrenaline and adrenaline were preserved in heparin plus glutathione (1.5% w/v). Blood samples were immediately centrifuged for 10 min at 3000 rpm at 4°C. Plasma was

transferred into microtest tubes, rapidly frozen in liquid nitrogen and stored at −70°C until further analysis. Plasma FFA concentrations were measured with the NEFA C kit (99475409, WAKO, Neuss, Germany), plasma glycerol concentrations were measured with a glycerol kit (148270, Boehringer, Mannheim, Germany) and plasma β-hydroxybutyrate concentrations were measured according to the method of Moore *et al*,[20] all on a Cobas-Fara centrifugal analyser (Roche Diagnostica, Basel, Switzerland). Plasma insulin concentrations were determined with a double-antibody radio immunoassay (Insulin RIA 100, Pharmacia, Uppsala, Sweden) and plasma noradrenaline and adrenaline concentrations by high performance liquid chromatography.[21] Standard samples with known concentrations were included in each for quality control.

Data analysis

All data are presented as mean±s.e.m. Data for energy expenditure were adjusted for FFM for group comparison.[22]

The effect of lipid heparin infusion between obese and lean subjects was analysed with a two-way repeated measurements ANOVA. *Post-hoc* testing was done with Student's unpaired *t*-test. A *P*-value smaller than 0.05 was regarded as statistically significant.

Results

At baseline, plasma FFA and glycerol levels were similar in both groups. Lipid heparin infusion significantly increased FFA and glycerol concentrations (both $P<0.001$; Figure 1). These increases were not significantly different between groups (obese *vs* lean, ΔFFA: 301±47 *vs* 332±27 μmol/l, NS; Δglycerol: 170±8 *vs* 151±11 μmol/l, NS).

Baseline energy expenditure was significantly higher in the obese (Table 2). After adjustment for FFM, baseline energy expenditure was similar in normal weight and overweight men (Figure 2). Energy expenditure significantly increased ($P<0.001$) during lipid heparin infusion. The increases were similar in both groups (obese *vs* lean: 0.34±0.08 *vs* 0.40±0.08 kJ/min, NS). RER was comparable between groups at baseline and decreased significantly

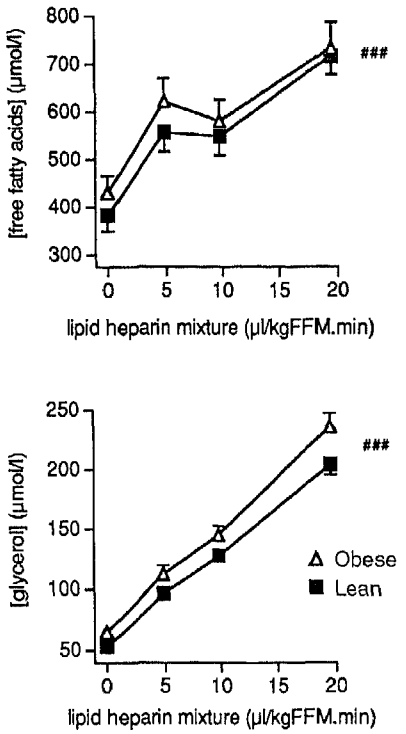

Figure 1 Plasma FFA and glycerol concentrations before and during the infusion of a lipid heparin mixture in 13 obese and 11 lean men. Values are mean±s.e.m. Repeated measurements ANOVA for treatment; $^{###}P<0.001$.

($P<0.001$) during lipid heparin infusion (Figure 2). Lipid oxidation was similar in both groups at baseline. During lipid heparin infusion, lipid oxidation increased significantly ($P<0.001$) and to a similar extent in obese and lean men (Δlipid oxidation: 19±5 *vs* 13±4 mg/min, NS, Table 2). Baseline carbohydrate oxidation did not differ between obese and lean men and significantly decreased ($P<0.05$) during lipid heparin infusion. The decrease in carbohydrate oxidation was similar between groups (obese *vs* lean: −29±12 *vs* −8±6 mg/min, NS, Table 2). Expressed as percentage of total energy expenditure, lipid and carbohydrate oxidation were similar in both groups at baseline.

Table 2 Energy expenditure and lipid and carbohydrate oxidation rates at rest and during lipid heparin infusion in lean and obese men

Parameter	*Baseline*	*4.9 μl/ kg_{FFM} · min*	*9.8 μl/ kg_{FFM} · min*	*19.6 μl/ kg_{FFM} · min*	*ANOVA for treatment*	*ANOVA for group × treatment*
Energy expenditure (kJ/min)					$P<0.001$	NS
Obese	5.97±0.19*	6.08±0.19*	6.07±0.20*	6.31±0.19*		
Lean	4.80±0.14	4.91±0.17	4.93±0.14	5.20±0.14		
Lipid oxidation (mg/min)					$P<0.001$	NS
Obese	64±7	73±9	71±9	83±6		
Lean	54±3	58±3	56±3	68±4		
Carbohydrate oxidation (mg/min)					$P<0.05$	NS
Obese	150±20	133±22	138±23	121±16		
Lean	114±9	113±10	119±9	106±10		

Results are mean±s.e.m. for 11 obese and 13 lean men. Unpaired *t*-test: $^{*}P<0.001$ obese *vs* lean.

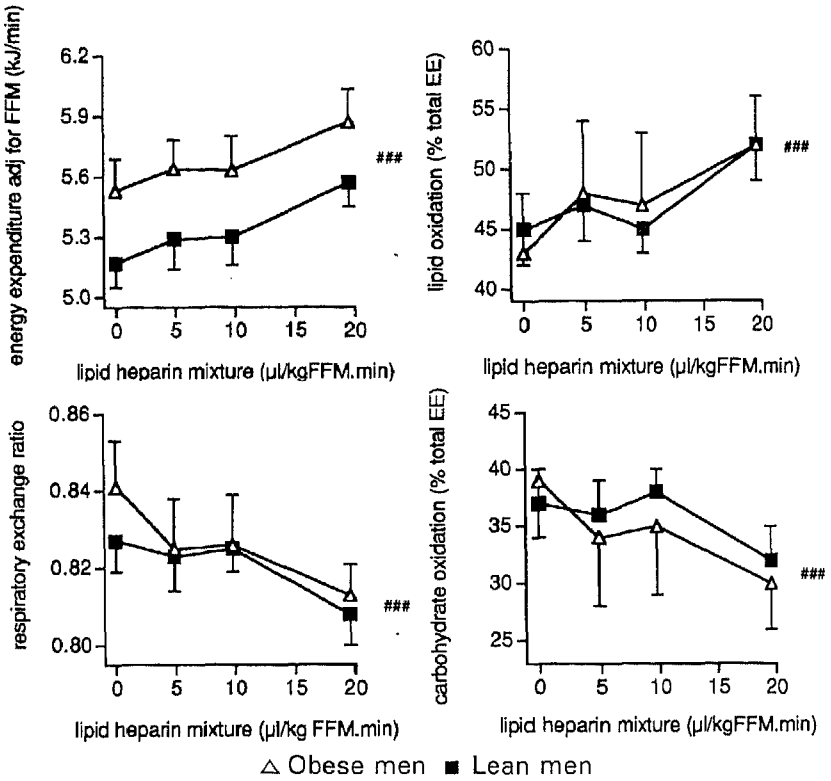

Figure 2 Energy expenditure adjusted for FFM, respiratory exchange ratio and lipid and carbohydrate oxidation rates expressed as percentage of total energy expenditure (EE) before and during the infusion of a lipid heparin mixture in 13 obese and 11 lean men. Values are mean±s.e.m. Repeated measurements ANOVA for treatment; $^{\#}P<0.05$, $^{\#\#\#}P<0.001$.

During lipid heparin infusion, lipid oxidation increased and carbohydrate oxidation decreased similarly in both groups (Figure 2).

Baseline plasma insulin levels were significantly higher in obese compared to lean men, but did not change during lipid heparin infusion in both groups (Table 3). Plasma β-hydroxybutyrate concentrations were similar at baseline and significantly increased ($P<0.001$) during lipid heparin infusion. However, the increase was significantly reduced in obese compared to lean men (Δβ-hydroxybutyrate: 55±26 *vs* 163±31 µmol/l, $P<0.05$, Table 3). Plasma noradrenaline and adrenaline concentrations did not differ significantly

Table 3 Plasma insulin, β-hydroxybutyrate, noradrenaline and adrenaline and concentrations at rest and during lipid heparin infusion in lean and obese men

Parameter	*Baseline*	*4.9 µl/ kg_{FFM} · min*	*9.8 µl/ kg_{FFM} · min*	*19.6 µl/ kg_{FFM} · min*	*ANOVA for treatment*	*ANOVA for group×treatment*
Insulin (mU/l)					NS	NS
Obese	19.4±3.5	19.0±3.2	19.3±3.3	19.7±3.7		
Lean	7.2±0.8	7.2±0.9	7.0±0.8	6.9±0.7		
β-Hydroxybutyrate (µmol/l)					$P<0.001$	$P<0.5$
Obese	104±12	96±35	98±22	150±34*		
Lean	113±20	140±26	174±35	277±43		
Noradrenaline (nmol/l)					$P<0.01$	NS
Obese	2.26±0.24	2.03±0.23	2.00±0.29	1.90±0.25		
Lean	2.09±0.22	1.91±0.16	1.90±0.19	1.79±0.21		
Adrenaline (nmol/l)					NS	NS
Obese	0.21±0.02	0.18±0.02	0.18±0.02	0.19±0.02		
Lean	0.28±0.04	0.31±0.05	0.30±0.04	0.30±0.04		

Results are mean±s.e.m. for 11 obese and 13 lean men. Unpaired *t*-test: $^{*}P<0.05$ obese *vs* lean.

between groups at baseline. During lipid heparin infusion, plasma noradrenaline levels significantly decreased ($P < 0.01$) and plasma adrenaline levels were unchanged in both groups (Table 3).

Discussion

The aim of the present study was to determine whether a certain increase in plasma FFA levels leads to similar increases in lipid oxidation and energy expenditure in obese and lean men. Plasma FFA levels were raised by infusing a lipid heparin mixture. Heparin was added to promote lipoprotein lipase activity and thus induce the hydrolysis of endogenous and exogenous triglycerides to FFA and glycerol. The increases in plasma FFA concentrations were similar in obese and lean men. Furthermore, the increases in lipid oxidation and thermogenesis were comparable between groups. Therefore, these data suggest that obese and lean men similarly increase their lipid oxidation and thermogenesis rates in response to a certain increase in plasma FFA levels.

The increase in energy expenditure during lipid heparin infusion in our study was comparable with that found by Thiebaud *et al*[23] in lean men. Jung *et al*[24] examined both obese and lean subjects, who received an i.v. bolus of heparin with or without concomitant lipid infusion, but no two subjects received the same amount of lipids. No difference was found in the response between obese and lean subjects plotting the increase in FFA level against the increase in energy expenditure, which is in accordance with our data. In contrast, Kjekshus *et al*[25] found no changes in O_2 consumption, and thus in thermogenesis, during lipid heparin infusion. However, this could be due to methodological problems, since O_2 consumption was calculated from CO_2 output and volumetric changes produced by respiration. Our increase in lipid oxidation after a certain increase in plasma FFA levels was comparable with the findings of Kleiber *et al*[26] in lean subjects and Golay *et al*[27] in obese subjects.

In order to exclude the possibility that increased SNS activity rather than increased FFA concentration induced the increases in energy expenditure and lipid oxidation, plasma noradrenaline and adrenaline levels were measured. Plasma adrenaline levels did not change and plasma noradrenaline levels even slightly decreased during lipid heparin infusion in both groups. This is in accordance with the findings of Jung *et al*,[24] who reached much higher plasma FFA concentrations in his experiment. This suggests that no additional SNS stimulation occurred during these tests. Plasma insulin levels, which inhibit FFA release from adipose tissue, changed neither in obese nor in lean men during lipid heparin infusion, which is in accordance with the findings of Thiebaud *et al*[23] The increase in energy expenditure and lipid oxidation is therefore likely to be induced directly by the increased FFA availability. Furthermore, a recent study from our laboratory showed that inhibition of lipolysis, and thus a lower plasma FFA availability, was accompanied by a smaller increase in energy expenditure and lipid oxidation during β_1-adrenoceptor stimulation.[28] This suggests that FFA availability may be a limiting factor for increasing lipid oxidation and energy expenditure.

An increase in plasma FFA levels does not only lead to increases in lipid oxidation and thermogenesis, but also to an increase in ketone body production in the liver. Furthermore, elevated plasma insulin concentrations seem to restrain FFA-induced ketogenesis.[29] This is in line with our findings, showing that elevated insulin concentrations in the obese were associated with a smaller increase in plasma β-hydroxybutyrate concentrations during lipid heparin infusion. Using the data of Keller *et al*,[29] it can be calculated that total lipid oxidation rates might be overestimated by ~30% if O_2 consumption is not corrected for the amount of O_2 needed for ketone body production. In our study, the increases in plasma β-hydroxybutyrate concentration were used to estimate the increases in ketone body production during lipid heparin infusion. It was found that the increase in lipid oxidation corrected for ketone body production did not significantly differ from that without correction in both groups. The larger increase in plasma β-hydroxybutyrate concentration in the lean therefore did not confound their increase in lipid oxidation, which remained comparable with that in the obese.

In this study, a certain raise in plasma FFA levels was accompanied by similar raises in lipid oxidation and thermogenesis in obese and lean men. Comparable results were found in a study in which we infused the selective β_1-adrenoceptor agonist dobutamine. In this study, obese and lean men showed similar increases in plasma FFA levels, lipid oxidation and energy expenditure in response to dobutamine.[30] Both studies suggest that obese men are capable of increasing their lipid oxidation and energy expenditure rates to the same extent as their lean counterparts. Furthermore, they provide no evidence for a difference in oxidative capacity in skeletal muscle between obese and lean subjects. The reduced increase in lipid oxidation in obese men during non-selective β-adrenergic stimulation, as found by Blaak *et al*,[10] or during selective β_2-adrenergic stimulation, as found by Schiffelers *et al*,[31] might therefore be explained by a reduced increase in plasma FFA concentration.

In conclusion, these data suggest that a certain increase in plasma FFA concentration leads to similar increases in energy expenditure and lipid oxidation in obese and lean men. The accumulation of fat in obese subjects may therefore be more likely to be due to a defect in adipose tissue lipolysis than a defect in lipid oxidation.

Acknowledgements

The authors wish to thank Joan Senden and Jos Stegen for their technical support during analysis of the blood samples. This study was supported by a grant from the Netherlands Organisation for Scientific Research (NWO), grant 903-39-138.

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